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EFFECTS OF pH AND CALCIUM NCENTRATION OF AMBIENT WATER ON CALCIUM DYNAMICS IN BROOK TROUT *SALVELINUS FONTINALIS*

Report No 83-463-K

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ABSTRACT

Juvenile brook trout, *Salvelinus fontinalis*, were maintained in waters of pH 5.3 or 6.5 and calcium concentrations of 5 or 40 mg/L to determine the effects of these factors on fish growth and calcium dynamics. Growth of brook trout, was reduced in waters of low pH (5.3 versus 6.5) and low calcium concentration (5.0 versus 40 mg Ca/L). However, only calcium concentration significantly affected calcium dynamics of the experimental fish. Calcium concentrations were reduced in skin and fins of trout in low calcium water. These fish also retained less dietary calcium and deposited less labelled calcium in bone than fish in high calcium water.

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740634-541-090	837.3	December 30, 1983	83-463-K

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To Mr. F.J. Kee
Director of Research

EFFECTS OF pH AND CALCIUM CONCENTRATION OF AMBIENT WATER ON CALCIUM DYNAMICS IN BROOK TROUT, *SALVELINUS FONTINALIS*

D.W. Rodgers

INTRODUCTION

The apparent decline of fish populations in acid-stressed waters is among the most visible effects of acidic deposition on the aquatic environment (Pfeiffer and Festa, 1980; NRCC, 1981). Generally, the effects of environmental acidification on fish are observed in a range of pH (5.5-6.5) above that which is acutely toxic (Fromm, 1980; Spry et al. 1981), and are manifested by changes in age structure of stressed fish populations. These changes, particularly the reduction or absence of younger year classes, are indicative of reproductive failure within the population (NRCC, 1981).

Although the effects of recent acidification on fish populations may be apparent, the mechanisms through which environmental acidification affects fish are less well defined. In acute exposure to low pH (<4.5), as in spring snowmelt, mortality appears to be related to failure of ionic regulation (Leivestad and Muniz, 1976). However, teleosts generally tolerate long term exposure to pH as low as 5.0 without apparent effects on ion and osmotic concentrations (Fromm, 1980). Thus, the chronic effects of pH on fish do not appear to result solely from osmoregulatory failure.

Calcium ions are central to a myriad of biological functions, ranging from muscle contraction to maintenance of biological membranes (Dacke, 1979). Female white sucker *Catostomus commersoni*, from Lake George an acid-stressed lake in the Canadian Shield do not display the normal prespawning elevation in serum calcium levels and do not reproduce successfully (Beamish et al. 1975). Calcium concentrations of centra of vertebrae of suckers from this lake are reduced, while manganese concentrations are elevated (Fraser and Harvey, 1982). These fish also have shorter caudal vertebrae and some fish have deformed caudal fins. In laboratory studies, both urinary excretion of calcium ions and loss of surface

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bound calcium from gills increase at low pH (McDonald and Wood, 1981; McWilliams, 1983). These observations from both field and laboratory thus indicate that interference with calcium regulation is one of the chronic effects of environmental acidification on fish.

The objective of this study was to determine the effects of ambient pH and calcium concentration on calcium dynamics in brook trout, *Salvelinus fontinalis*, an important sports fish, native to waters of Ontario sensitive to acidic deposition (Minns, 1981). Changes in calcium dynamics were studied to characterize their utility in explaining the effects of chronic acidification on fish.

Knowledge of the mechanisms through which environmental acidification affects fish is central to understanding the impact of acidic deposition on fish populations. A thorough understanding of the physiological changes characteristic of acid-stressed fish may enable stressed fish populations to be identified prior to reproductive failure and provide a scientific basis for evaluation of short-term remedial measures such as liming of lakes.

MATERIALS AND METHODS

Juvenile brook trout were obtained from a commercial hatchery (Glennwood Trout Hatchery, Ashburn, Ontario). Prior to experiments, fish were maintained in 400 L tanks supplied with dechlorinated Toronto water (15 ±1°C, pH 7.5, 40 mgCa/L) and fed to satiation with a commercial trout diet three times each week. Laboratory photoperiod was regulated to correspond to natural light regime, and increased from ≈8 h L - 16 D in December to ≈12 h L - 12 h D in March.

For the experiments, trout (initial weight ≈20±5 g) were transferred to and maintained in 20 L plastic tanks which were contained in a temperature regulated water bath (15 ±1.0°C). Tanks were aerated with compressed air and supplied with 7.5 L/h of water of controlled calcium concentration (5 or 40 mg Ca/L) and pH (5.3 or 6.5), with two replicate tanks for each of the four combinations of pH and calcium. The calcium concentrations of approximately 5 mg Ca/L and pH of 5.0-5.5 are typical of acid stressed lakes (NRCC, 1981).

The trout were fed a specially prepared, low calcium diet (Appendix 1) for 3 weeks; fed this same diet labelled with ^{45}Ca for the next 4 weeks and then returned to the non-labelled, low calcium diet for the subsequent 4 weeks. Fish were fed a ration of 1.5% wet weight/d for 5 days (Monday-Friday) then not fed for the next 2 days. Fish were weighed and rations adjusted weekly.

Fish were transferred to experimental tanks at an initial density of 13 fish per tank. At 3, 10, 17 and 31 d after last feeding of labelled diet, a sample of 3 fish was removed at random from

each tank. These sampling periods are subsequently referred to as Week 0, Week 1, Week 2 and Week 4 respectively. Fish were killed by anesthetization in a tricane methane-sulphonate (MS222) solution (50 mg/L) weighed and frozen (-20°C) in individual plastic bags for 2-4 weeks until processed. Frozen fish were steamed in an autoclave for 3 min and then dissected to separate skin and fins, axial skeleton and remaining tissues. Tissue samples were ashed at 700°C to constant weight, dissolved in nitric acid (5 mL) and then made up to constant volume (25 mL) with distilled water for analysis of stable and radioactive calcium.

Dechlorinated Toronto tap water was deionized using a commercial deionizer (Culligan, charcoal filter and 30 cm two-bed strong base) and supplied to two head tanks. Within the head tanks, a pH tritiator regulated pH (using 0.01 M H_2SO_4 or NaOH as required) to maintain nominal pH's of either 5.3 or 6.5 in experimental tanks. A peristaltic pump delivered a salt solution containing sodium, potassium and calcium chloride to pH-regulated deionized water before delivery to experimental tanks. Nominal sodium and potassium concentrations were 4.0 and 0.5 mg/L respectively for all tanks; nominal calcium and chloride concentrations were 40 and 70 mg/L respectively for high calcium tanks and 5 and 10 mg/L respectively for low calcium tanks.

There was no mortality of experimental fish during the period of acclimation and feeding of experimental diet. Subsequent to the first sampling period (Week 0) a system failure resulted in complete mortality of fish in pH 6.5-low calcium tanks. In other tanks there was no mortality until the last week when two fish in one of the pH 5.3-high calcium tanks died. These fish displayed severe erosion of the caudal fin and caudal peduncle which apparently resulted from persistent aggression by the dominant fish in the tank. Fish in all tanks fed well during the acclimation period and feeding of labelled diet. After the third sampling period (Week 2) apparent appetite of fish in all tanks decreased, although all food was consumed. This decrease in appetite appeared to correspond to an increase in aggression among fish within the tank, suggesting that at this low density, (four fish per tank) fish became territorial and feeding of subordinate fish may have been suppressed.

Dissolved oxygen concentrations were always greater than 80% of air saturation and calcium concentrations maintained within 5% of nominal. Continuous recordings of pH in experimental tanks revealed diurnal variation in pH of approximately 0.3 and 0.2 pH units in pH 5.3 and 6.5 tanks respectively. These variations in pH apparently correspond to diurnal variations in rates of release of respiratory and excretory products by fish, with concomitant changes in buffering capacity of the experimental tanks. Actual pH was thus maintained within 0.3 pH units of the 5.3 nominal for fish in the low pH tanks and 0.2 pH units of the 6.5 nominal for fish in the high pH tanks. Ammonia levels

were measured periodically and were less than 1 mg/L. Similarly, other metal and anion concentrations in test waters (Appendix 2) were within water chemistry limits suggested as conducive to optimum health of cold-water fishes (Wedemeyer and Yasutake, 1977).

The specially prepared low calcium diet was obtained from the Department of Nutrition, University of Guelph. The dry diet was mixed with 50% distilled water, extruded in a food mixer, lyophylized and kept frozen in plastic bags until used. Diet labelled with with ^{45}Ca was prepared in the same way but with approximately 40 MGq of ^{45}Ca labelled $CaCl_2$ (specific activity 670 MBq/mg Ca - New England Nuclear, Montreal, Quebec) added to the aqueous solution before mixing with 1.5 kg of diet. Calcium concentrations of labelled and non-labelled diets were 5.37±0.26 and 5.27±0.23 mg Ca/g dry weight respectively (mean ± SD, n = 15). The activity of the labelled diet at the time of first sampling was 1.70±0.05 k Bq/g (n = 14).

Routine determinations of calcium, pH and dissolved oxygen of water in the test tanks were made using standard methods (APHA et al, 1981). Routine calcium measurements obtained by tritiation with EDTA agreed well with values obtained for the same samples using atomic absorption spectrometry.

Calcium in tissue digests was determined by atomic absorption spectrometry (Ontario Hydro Research Division Report CN83-634-K; CN83-706-K; CN83-822-K and CN83-870-K). ^{45}Ca was determined by scintillation counting of duplicate samples of acidified tissue ash using a Packard Tri-carb Scintillation Counter (Model 300C) and Monophase 40 liquid scintillation cocktail (Ontario Hydro Research Division Report CM82-2493-K). Results are expressed as activity per mg Ca and are connected for background, quench and radioactive decay.

In water samples, anion concentrations were determined by ion chromatography and colorimetry (Ontario Hydro Research Division Report CE83-549-K), while Ca, Mg, Na, K and Zn were determined by atomic absorption spectrometry (AAS) and Cd, Cu, Ni and PH were determined by AAS using a graphite furnace (Ontario Hydro Research Division Report CE83-550-K).

Growth rates were determined from the change in weight of all fish in the tank over the period that the fish were fed labelled diet. During this period constant numbers of fish were maintained in all tanks and fish fed avidly. Growth rates were determined from the difference between initial and final weights over the period and expressed as % increase/d. The mean retention of labelled dietary calcium (%) was determined from the ratio of the quantity of labelled calcium consumed by fish within the tank (amount consumed (g) multiplied by mean activity of labelled diet (k Bq/g)) to the activity of fish in the tank (weight of all fish in tank at week 0 sample (g) multiplied by the mean specific activity of fish sample at week 0 (k Bq/g)).

Results of these experiments were analyzed using the appropriate analysis of variance models. Cell means were compared using the least significant difference (LSD) method if the F ratio was significant ($P \leq 0.05$). In all statistical tests of significance, differences between cell means were considered significant if $P \leq 0.05$. Statistical transformation of data was not required as visual inspection of residual and normal probability plots did not reveal apparent violations of statistical assumptions of normality and homogeneity of variance using raw data. Data for individual fish are contained in file No 837.3.

RESULTS

Over the 4 weeks the trout were fed labelled diet, growth rates of the trout under different treatments varied more than two-fold (Figure 1). Both pH and calcium concentration significantly affected growth rate with no apparent interaction between factors. The average growth rate of fish maintained in water of high calcium concentration (1.38±0.08% wet wt/d(mean ± SE)) was significantly greater than that of fish kept in water of low calcium concentrations (0.97 ± 0.08% wet wt/d). Similarly, the growth rate of fish at pH 6.5 was significantly higher than that of fish at pH 5.3 (1.47 ± 0.08 and 0.88 ± 0.08% wet wt/d, respectively).

Ash content of the brook trout changed slightly but significantly over the sampling period; however, this variation was not related to either pH or calcium concentration (Figure 2). Mean ash content of fish at Week 0 (2.19 ± 0.05% wet wt) was significantly lower than that at all other sampling periods (2.52 ± 0.04%, 2.52 ± 0.04% and 2.41 ± 0.04% wet wt at Weeks 1, 2 and 4 respectively). Variation in ash content among other sampling periods was not statistically significant.

As the pattern of variation in calcium concentration differed among tissues, changes in calcium concentration of each tissue compartment are examined separately. Calcium concentration of the axial skeleton was stable throughout the experiment (339 ± 14 mg Ca/g Ash) and was not significantly affected by pH, calcium concentration or duration of the experiment (Figure 3a). In contrast, the calcium concentration of the skin and fins was significantly affected by the calcium concentration of the ambient water but not by pH or experimental duration (Figure 3b). In fish samples at Week 0, the mean calcium concentration of skin and fins of trout in high calcium water (304 ± 2.2 mg Ca/g ash) was significantly higher than that of fish in low calcium water (294 ± 2.1 mg Ca/g ash). Among fish maintained in water with the high calcium concentration (pH 5.3 and 6.5), there was a significant temporal variation in calcium concentration of remaining tissues, with mean calcium concentration at Week 0 (70.3 ± 4.0 mg Ca/g ash) significantly lower than that at week 3 (88.6 ± 4.0 mg Ca/g ash) (Figure 3c). However, at weeks 2 and 4, average calcium concentrations of

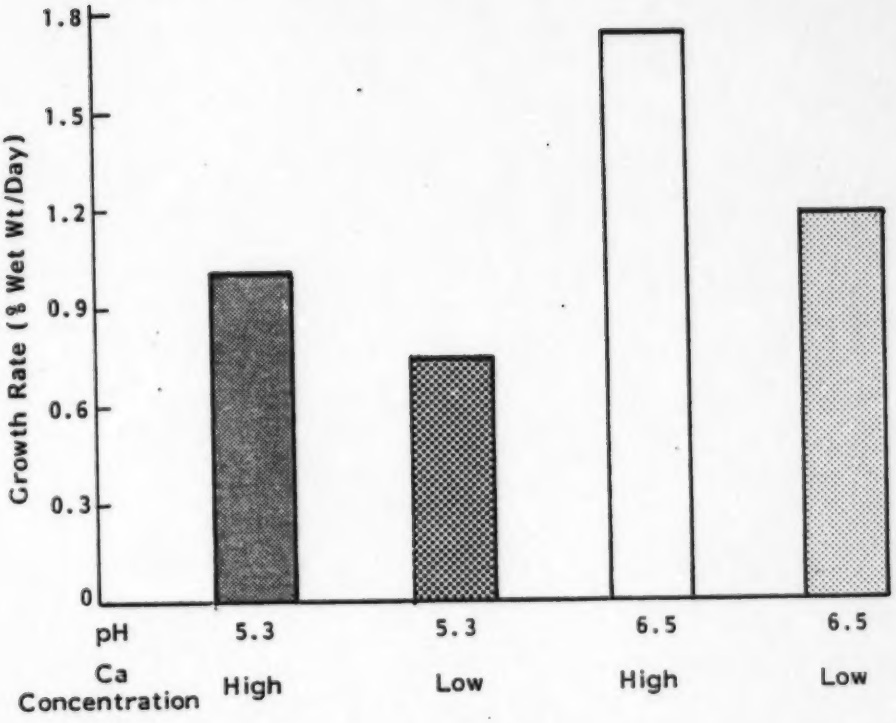

FIGURE 1
GROWTH RATE OF EXPERIMENTAL TROUT

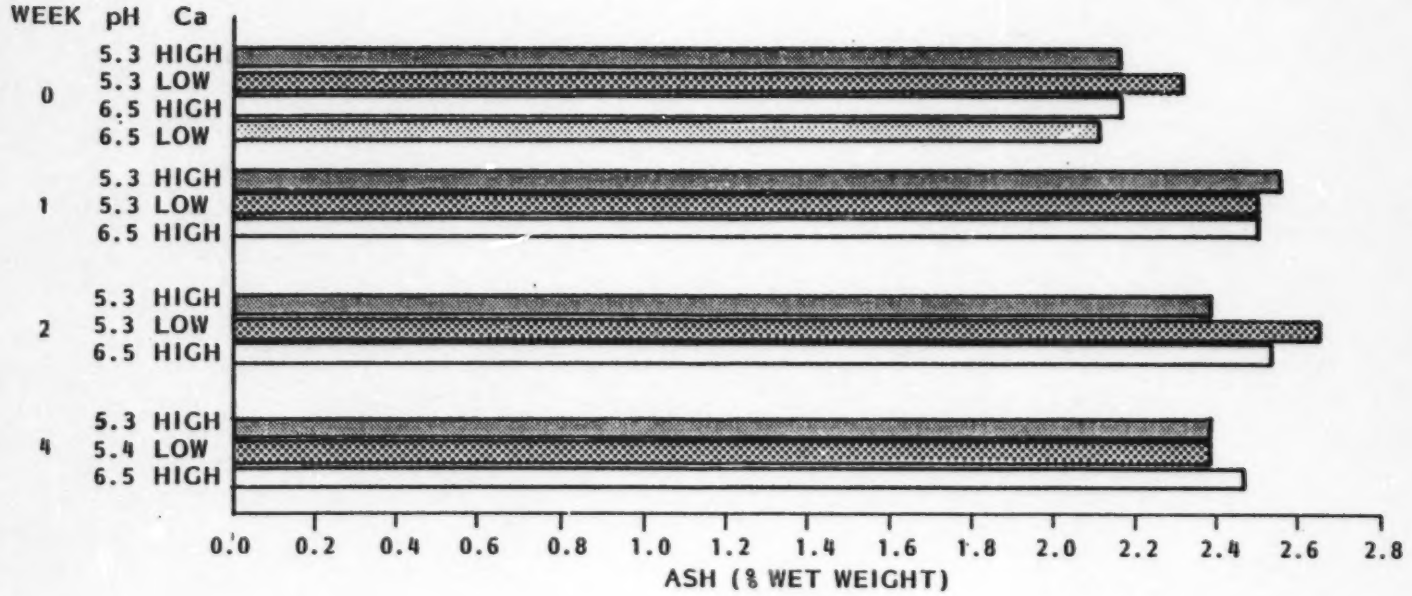

FIGURE 2

ASH CONTENT OF EXPERIMENTAL TROUT

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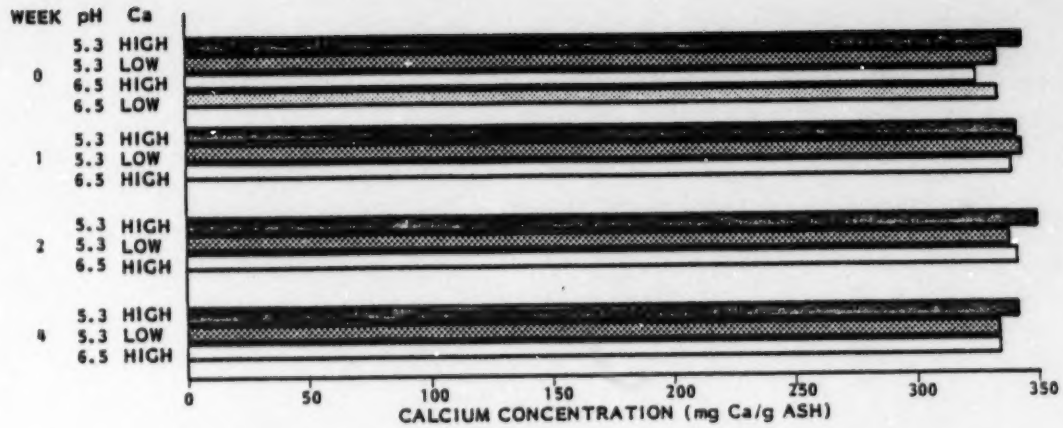

FIGURE 3A
CALCIUM CONCENTRATION OF AXIAL SKELETON OF EXPERIMENTAL TROUT

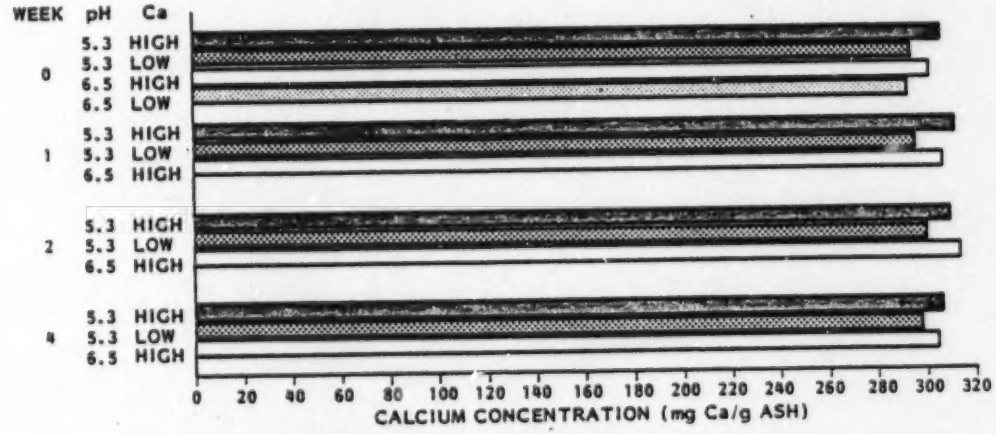

FIGURE 3B
CALCIUM CONCENTRATION OF SKIN AND FINS OF EXPERIMENTAL TROUT

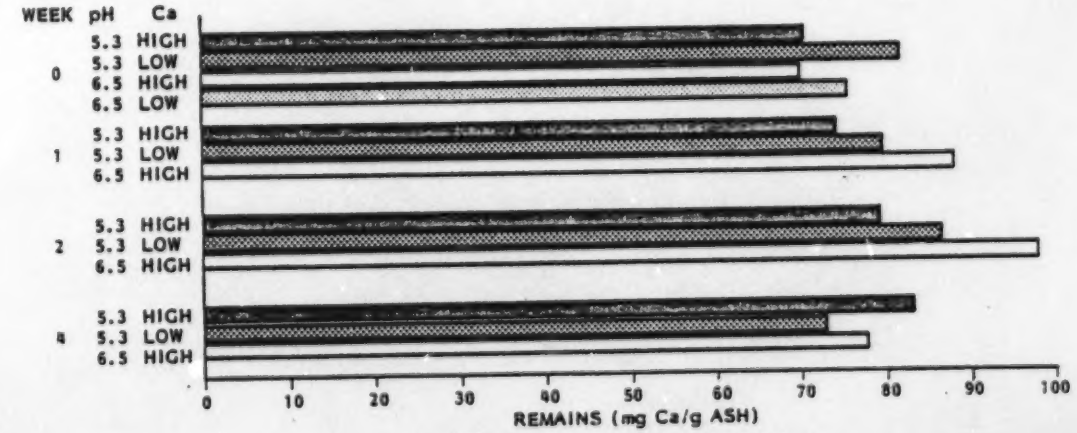

FIGURE 3C
CALCIUM CONCENTRATION OF REMAINING TISSUES OF EXPERIMENTAL TROUT

remaining tissues (80.9 ± 4.3 and 79.6 ± 4.1 mg Ca/g ash, respectively) were not significantly different from either Week 0 or Week 3. Among fish at pH 5.3 (high and low ambient calcium concentrations), calcium concentration of remaining tissues was not significantly affected by ambient calcium concentration or duration of experiment.

Changes in the proportion of total calcium in each of the tissue compartments largely paralleled changes in calcium concentration. At Week 0, the proportion of total calcium in skin and fins of fish maintained in water of high calcium concentration (48.9 ± 1.1%) was significantly greater than that of fish in water of low calcium concentration (45.5 ± 1.1%). The proportion of total calcium in each of the tissue compartments changed slightly but significantly over the duration of the experiment (Figure 4a, b and c). Among fish maintained at pH 5.3, the proportion of total calcium in the axial skeleton during Weeks 2 and 4 (41.9 ± 0.7 and 40.6 ± 0.8%, respectively) was significantly higher than that at Week 0 (38.7 ± 0.8). The proportion of total calcium in the axial skeleton at Week 1 (39.5 ± 0.8%) was significantly less than Week 2 but not significantly different from Weeks 0 and 4. Among fish at pH 5.3, changes in the proportion of total calcium in the remaining tissues largely reciprocated changes in the axial skeleton. At Week 0, a significantly higher proportion of total calcium was in remaining tissues (15.4 ± 0.8%) than at Weeks 1, 2 and 4 (12.4 ± 0.8%, 13.3 ± 0.7% and 12.8 ± 0.7% respectively). Among fish maintained in water of high calcium concentration, the proportion of total calcium in axial skeleton at Week 0 (37.3 ± 0.8%) was significantly lower than in Weeks 1, 2 and 4 (39.8 ± 0.8%, 40.9 ± 0.8% and 40.8 ± 0.8%, respectively) which were not significantly different from each other. For these fish, the changes in bone were offset by a reciprocal trend in the proportion of calcium in skin and fins, with levels at Weeks 0 and 1 (48.9 ± 0.8% and 47.2 ± 0.8%) significantly higher than at Week 2 (44.6 ± 0.8%). The proportion of calcium in skin and fins at Week 4 (46.4 ± 0.8%) was significantly higher than Week 0 but not significantly different from Weeks 1 and 2.

Apparent retention of ^{45}Ca in the labelled diet was significantly affected by calcium concentration but not by pH (Figure 5). Mean retention of labelled calcium by fish in water of high calcium concentration (38.0 ± 1.8%) was significantly greater than retention of ^{45}Ca by fish in water of low calcium concentration (30.7 ± 1.8%).

Trends in variation of specific activity of each tissue compartment are examined separately as changes in the distribution of radiolabelled calcium differed among tissues. The specific activity of the axial skeleton was significantly affected by ambient calcium concentration but not by pH or experimental duration (Figure 6a). At Week 0, the mean specific activity of axial skeleton of trout in water of high calcium concentration

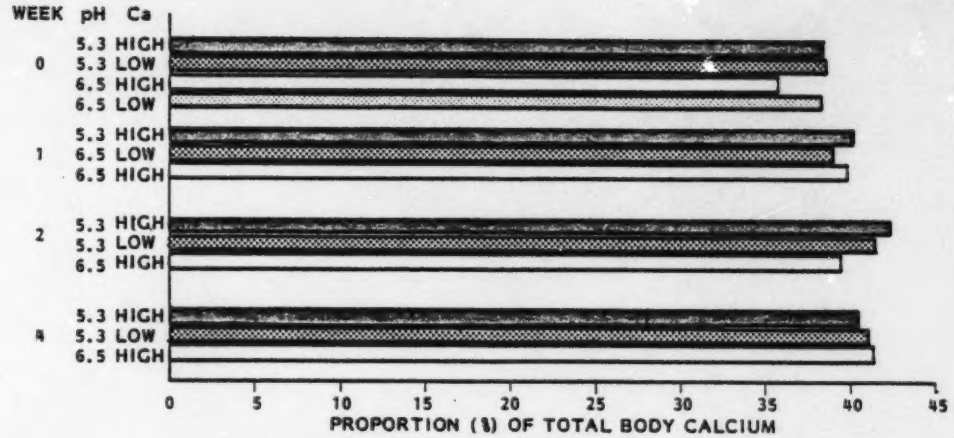

FIGURE 4A

PROPORTION (%) OF TOTAL CALCIUM IN AXIAL SKELETON
OF EXPERIMENTAL TROUT

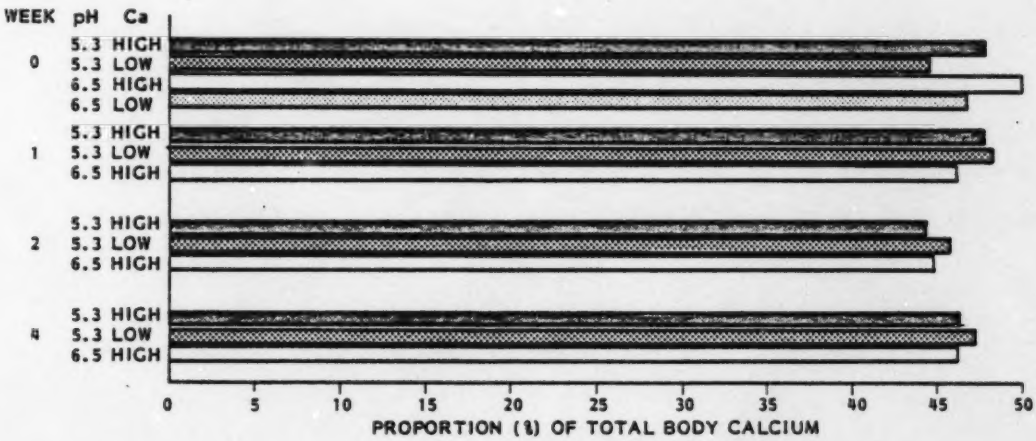

FIGURE 4B

PROPORTION (%) OF TOTAL CALCIUM IN SKIN
AND FINS OF EXPERIMENTAL TROUT

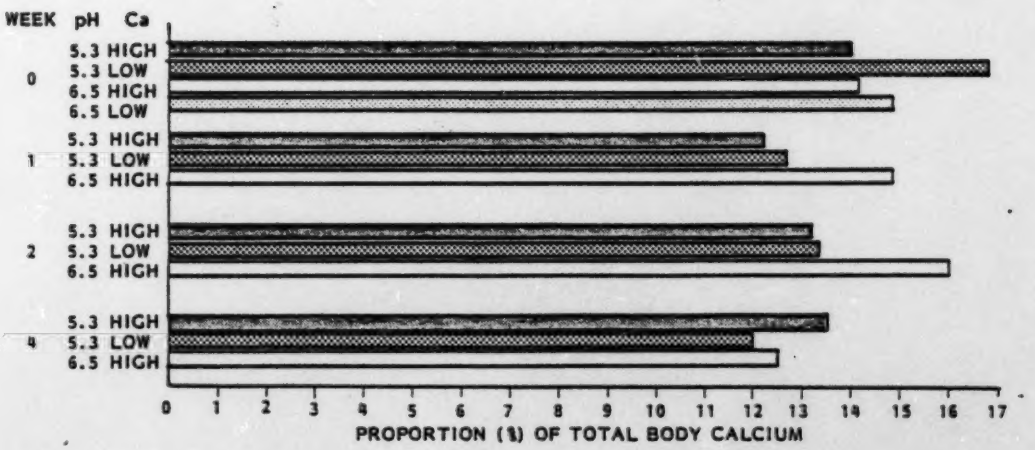

FIGURE 4C

PROPORTION (%) OF TOTAL CALCIUM IN
REMAINING TISSUES OF EXPERIMENTAL TROUT

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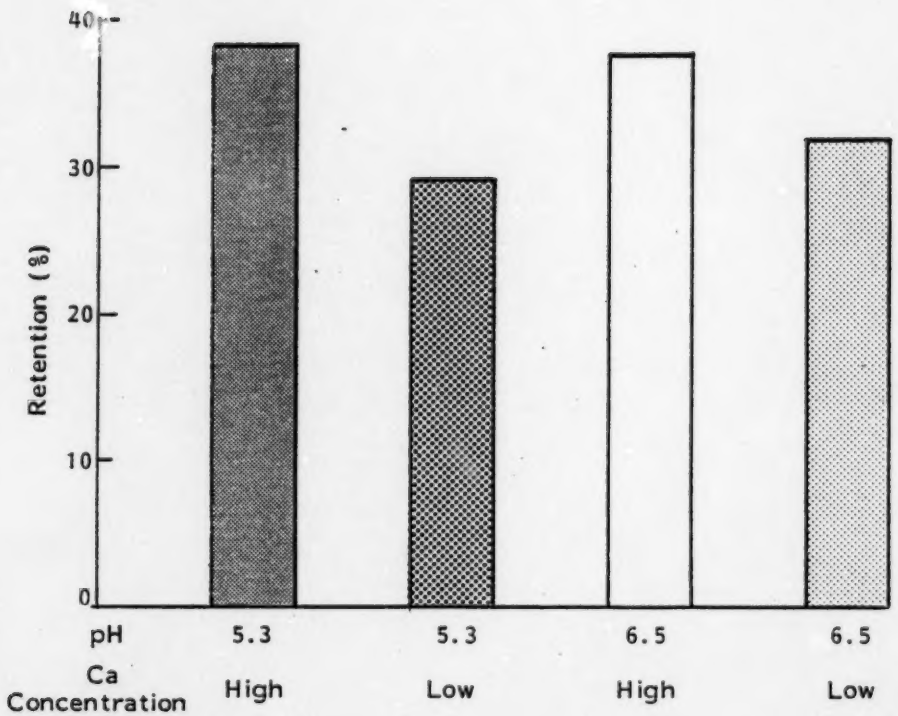

FIGURE 5

APPARENT RETENTION (%) OF DIETARY ^{45}Ca
BY EXPERIMENTAL TROUT

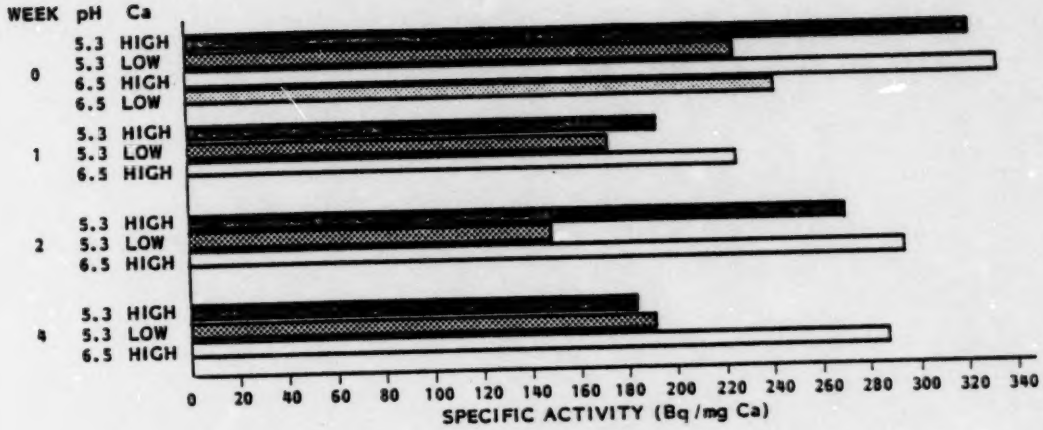

FIGURE 6A
SPECIFIC ACTIVITY OF AXIAL SKELETON
OF EXPERIMENTAL TROUT

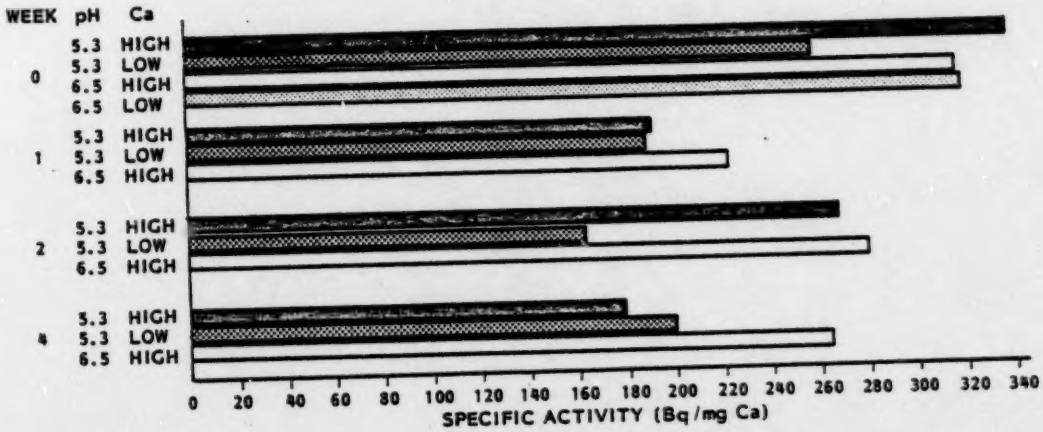

FIGURE 6B
SPECIFIC ACTIVITY OF EXPERIMENTAL TROUT SKIN AND FINS

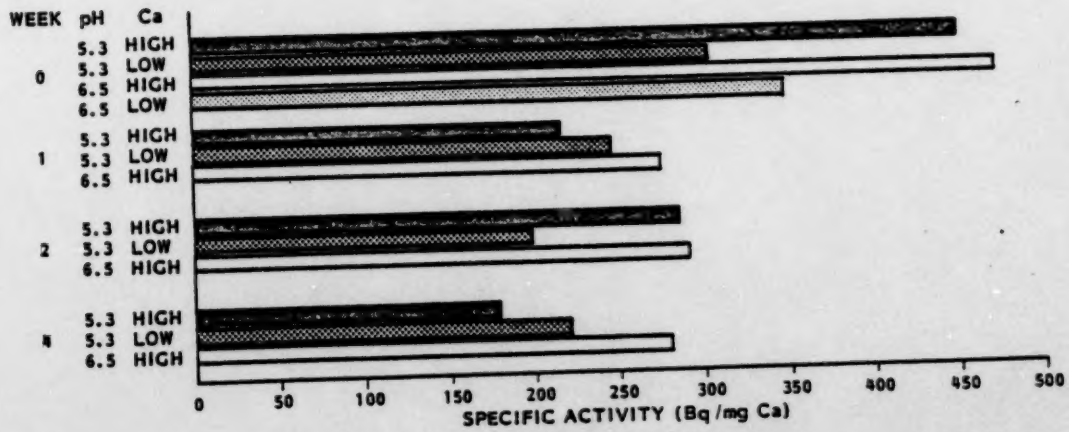

FIGURE 6C
SPECIFIC ACTIVITY OF REMAINING TISSUES OF EXPERIMENTAL TROUT

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(328 ± 24 Bq/mg Ca) was significantly greater than that of fish from low calcium water (233 ± 24 Bq/mg Ca). A similar pattern was seen in the remaining tissues at Week 0 with significantly higher specific activities observed in fish from water of high calcium concentrations (mean activities of 462 ± 37 and 327 ± 37 Bq/mg Ca in waters of high and low calcium concentration, respectively). In addition, the specific activity of the remaining tissues decreased significantly with time after last feeding of labelled diet (Figure 6c). Mean specific activity of the skin and fins declined significantly with time but was not significantly affected by pH or calcium concentration (Figure 6b).

The differences in mobility of calcium within specific tissue compartments are reflected by the changes in specific activity with time. At Week 0, the mean specific activity of remaining tissues was significantly greater than that of skin and fins which in turn had a significantly higher specific activity than the axial skeleton. In Weeks 1 and 2, the mean specific activity of the remaining tissues was still significantly higher than that of both skin and fins, and axial skeleton but the average specific activities of these two tissues were not significantly different. At Week 4, differences in specific activity among tissues were not statistically significant.

DISCUSSION

Both pH and calcium concentration significantly affected growth of brook trout. However, only calcium concentration significantly influenced calcium dynamics in the experimental fishes. These results thus indicate that pH by itself may have relatively little effect on calcium dynamics and skeletal growth in salmonids. Conversely, ambient calcium concentration had a pronounced effect on calcium dynamics in fish. Apparent anomalies in calcium regulation of fish in acid-stressed waters may thus result from factors other than pH alone, possibly the combined stresses of low pH and elevated metal concentrations.

The consistent reduction in growth rate of fish at pH 5.3 relative to fish at pH 6.5 demonstrated that low pH reduced growth of brook trout fed fixed rations. Previous experimental studies of the effects of pH on fish growth reported either a reduction in growth at low pH or no effect of pH on growth. For alevins and adult brook trout fed *ad libitum*, Menendez (1976) reported that fish maintained at pH 4.5 and 5.0 grew more slowly than fish at pH 7.0. However, growth rates of yearling brown trout, *Salmo trutta*, fed fixed rations (2.9% body weight/day for 48 days) did not differ significantly among fish maintained at pH 6.26, 5.44 and 5.0 (Jacobsen, 1977). In the present study, fish consumed all food offered and the reduction in growth observed for brook trout maintained at pH 5.3 is not merely an artifact of reduced appetite. Unfortunately, at present, information concerning the

effects of pH on fish energetics is not sufficient to suggest the metabolic basis of reduced growth at low pH. Metabolic compensation to low pH may also be possible as Menendez (1976) noted that growth rates of adult brook trout at pH 5.0 and 7.0 were not significantly different in the last two months of his five month study. The differences in response of brook and brown trout to low pH may also reflect species specific differences in susceptibility.

The reduction in growth rate of trout maintained in water of low calcium concentration indicates that under the conditions tested, a low ambient concentration of calcium may limit fish growth. This result was unexpected as in previous studies with a number of fish species, dietary calcium concentrations ranging from 1 to 10 mg/g did not affect fish growth (Ogino and Takeda, 1976, 1978; Watanabee et al, 1980; Ichii and Mugiya, 1983), although a dietary calcium concentration of 15 mg/g was required for maximal growth in channel catfish, *Ictalurus punctatus* (Andrews et al, 1973). The above studies, however, were conducted in waters with a calcium concentration of approximately 20 mg/L. At this concentration, increased calcium uptake from water appears to compensate for reduced dietary uptake (Ophel and Judd, 1976; Ichii and Mugiya, 1983). Unfortunately, although Wedemeyer and Yasutake (1977) recommend an alkalinity of at least 20 mg $CaCo_3$/L as conducive to optimal health of cold-water fishes, additional studies comparing the growth of fish maintained in waters of different calcium concentrations are not presently available. The dietary calcium concentration (5 mg Ca/g dry wt) used in the present study is low relative to that of commerical trout feeds (>10 mg Ca/g) but is similar to the range of 1-4 mg Ca/g fresh wt reported for the diet of planktivorous fish (Berg, 1968). In addition, calcium concentrations of 4 mg/L or less are often reported for soft water lakes which are sensitive to acidification (NRCC, 1981). The present results suggest that in these environments with low calcium concentrations in both water and natural diet, calcium availability could limit the fish growth.

The small increase in ash content of fish from week 0 to weeks 1, 2 and 4 (2.2 to 2.5% ash) may reflect a slight increase in mineralization of the fish with time, but was not affected by pH or calcium concentration. The observed ash content is within the range previously reported for salmonids, 2.6 ± 0.02% for brown trout, *Salmo trutta*, (Elliot, 1976) and 1.8 to 2.6% for rainbow trout (Ogino and Takeda, 1978). Similarly, the slight increase with time in the proportion of total calcium in the axial skeleton of the trout may reflect increasing ossification of the skeleton of the fish with time. The proportions of total calcium in axial skeleton (35-41%) and skin and fins (44-50%) are similar to that previously reported for teleosts (Simkiss, 1974; Dacke, 1979).

The differences in calcium dynamics among the tissue compartments reflect differences in the physiological and metabolic functions

of calcium in individual tissues. Calcium in the axial skeleton is predominantly utilized in the structural matrix of bone (Dacke, 1979). Generally, calcium in bone is relatively stable with only 5 to 10% of the calcium readily exchangeable with tissue fluids (Simkiss, 1974). The conservative behaviour of calcium in bone was confirmed in the present experiments in which neither pH nor calcium concentration significantly affected calcium concentration of the axial skeleton. Similarly, the specific activity of the axial skeleton did not change significantly over the 4 weeks after feeding labelled diet. In contrast, the specific activities of both remaining tissues and skin and fins decreased significantly with time, reflecting mobilization or dilution of calcium within these pools. The observed calcium concentration of the axial skeleton of brook trout ($\approx$340 mg Ca/g ash) is similar to mean concentrations of 334-343 mg Ca/g ash reported for bone of goldfish, *Carassius auratus* (Ophel and Judd, 1976).

The lower specific activity of both axial skeleton and remaining tissues of trout from low calcium water at week 0 corresponds well with the lower apparent retention of radiolabelled dietary calcium by these fish. This lower apparent retention of dietary calcium may derive from a decreased rate of uptake of dietary calcium or an increased rate of calcium efflux by fish in water of low calcium concentration. In a series of experiments using ^{85}Sr as a tracer of calcium and strontium uptake by goldfish, Berg (1968) indicates that at equivalent dietary calcium concentrations, a greater proportion of total calcium uptake is derived from dietary sources in waters of low calcium concentrations. Information concerning the effects of ambient calcium concentration on calcium efflux of fish is limited, although Phillips et al (1957) indicate that a greater proportion of labelled dietary calcium is excreted by brook trout in low calcium water (5 mg/L) than in water of higher calcium concentration (50 mg/L). For a number of teleosts, efflux across the gills of both nonpolar molecules (tritiated water) and ions (Na^+, H^+ and Cl^-) increased at low ambient calcium concentrations (Ogawa, 1974; Isaia and Mansoni, 1976; McWilliams and Potts, 1978) presumably efflux of Ca^{++} ion is similarly increased at low ambient calcium concentrations. Existing evidence thus suggests that the observed lower apparent retention of dietary calcium by brook trout in water of low calcium concentration results from an increased rate of loss of calcium from the fish rather than reduced uptake of dietary calcium. The lower specific activity of axial skeleton of trout in low calcium water would result from less labelled calcium remaining available for deposition in bone. The reduced deposition of labelled dietary calcium did not affect total calcium concentration of the bone in the present short-term study; however, differences might become apparent in studies of a longer duration.

The lack of effect of pH on calcium dynamics seems contrary to field reports of reduced calcium concentrations in centra of vertebrae from white suckers caught in lakes of low pH and calcium concentration (Fraser and Harvey, 1982). However, in the present study, only pH and calcium concentrations were varied, while in the field, elevated concentrations of metals (Al, Cd, Ca, Pb, Mn, Ni and Zn) are frequencly associated with low pH (Spry et al. 1981). Significantly, Fraser and Harvey (1982) reported elevated concentrations of manganese as well as reduced calcium concentration in centra of suckers from the most acid lake, George Lake (pH 4.65). Manganese-deficient rainbow trout display abnormal curvature of the backbone and tail (Ogino and Yang, 1980), while in humans manganese poisoning has been suggested to alter calcium metabolism (Chandra et al. 1974). It is thus probable that the apparent interference in calcium regulation observed in fish from recently acidified waters results from interactions among low pH, low calcium concentrations and elevated metal concentrations. The nature of these interactions and the possibility of remedial actions have yet to be quantified.

SUMMARY

1. Juvenile brook trout were maintained in waters of pH 5.3 or 6.5 and calcium concentrations of 5 or 40 mg/L to determine the effects of these factors on fish growth and calcium regulation.

2. Growth of trout was significantly reduced by low pH and low calcium concentration.

3. Only calcium concentration significantly affected calcium dynamics of the trout. Fish in low calcium water had a significantly lower concentration of calcium in skin and fins, retained less labelled dietary calcium and deposited less labelled calcium in axial skeleton. Anomalies in calcium regulation of fish from acid-stressed lakes may thus be caused by factors other than low pH alone.

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APPENDIX 1

FORMULATION OF THE TEST DIET

	%
Casein	40
Gelatin	5
Wheat Gluten	5
Starch	15
α-Floc	9
Amino Acid Premix	1
Vitamin[1] Premix	2
Mineral[2] Premix	8
Fish Oil	15
	100%

1. Vitamin Mix as in Hilton et al. 1982 - Br.J.Nutr.
2. Mineral Mix as in Hilton et al. 1980 - J. Nutrition

   Minus - $CaHPO_4 2H_2O$
   $CaCO_3$

APPENDIX 2

METAL AND ION CONCENTRATIONS OF TEST WATERS, MARCH 14, 1983

(All concentrations in mg/L)

pH	5.3	5.3	6.5	6.5
$[Ca]^+$	Lo	Hi	Lo	Hi
F	<0.05	0.27	<0.05	0.23
Cl	14	91	12	73
NO_3	<0.2	<0.2	<0.2	<0.2
SO_4	0.79	0.84	0.81	0.86
NH_4	0.20	0.14	-	0.52
Ca	5.0	45	4.2	36
Mg	<0.02	<0.02	<0.02	<0.02
Na	2.3	2.7	5.0*	5.2*
K	0.54	0.60	0.49	0.56
Zn	0.08	0.08	0.08	0.09
Pb	0.002	0.005	0.002	0.002
Cd	0.001	<0.0001	<0.0001	<0.0001
Cu	0.003	0.005	0.007	0.008
Ni	<0.005	<0.005	<0.005	<0.005

*Higher Na concentration due to titration with NaOH to maintain pH 6.5.

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